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Redox activity of airborne particulate matter at different sites in the Los Angeles Basin

Arthur K. Cho[a,b], Constantinos Sioutas[a,c], Antonio H. Miguel[a], Yoshito Kumagai[a,d], Debra A. Schmitz[a,b], Manisha Singh[a,c], Arantza Eiguren-Fernandez[a], John R. Froines[a,e,*]

[a]*Southern California Particle Center and Supersite, Institute of the Environment, University of California Los Angeles, Los Angeles, CA 90095, USA*
[b]*Department of Molecular and Medical Pharmacology, School of Medicine, University of California Los Angeles, Los Angeles, CA 90095, USA*
[c]*Department of Civil and Environmental Engineering, University of Southern California, Los Angeles, CA 90089, USA*
[d]*Department of Environmental Medicine, Institute of Community Medicine, University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan*
[e]*Center for Environmental & Occupational Health, School of Public Health, University of California Los Angeles, Los Angeles, CA 90095-172, USA*

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Abstract

Epidemiologic studies have shown associations between ambient particulate matter (PM) and adverse health outcomes including increased mortality, emergency room visits, and time lost from school and work. The mechanisms of PM-related health effects are still incompletely understood, but a hypothesis under investigation is that many of the adverse health effects may derive from oxidative stress, initiated by the formation of reactive oxygen species (ROS) within affected cells. While the adverse effects from PM have historically been associated with the airborne concentration of PM and more recently fine-particle PM, we considered it relevant to develop an assay to quantitatively measure the ability of PM to catalyze ROS generation as the initial step in the induction of oxidative stress. This ability of PM could then be related to different sources, chemical composition, and physical and spatial/temporal characteristics in the ambient environment. The measurement of ROS-forming ability in relation to sources and other factors will have potential relevance to control of redox-active PM. If oxidative stress represents a relevant mechanism of toxicity from PM, the measurement of redox activity represents a first step in the elucidation of the subsequent downstream processes. We have developed an assay for PM redox activity, utilizing the reduction of oxygen by dithiothreitol which serves as an electron source. We have found that PM will catalyze the reduction of oxygen and have examined the distribution and chemical characteristics of the redox activity of PM fractions collected in different sites in the Los Angeles Basin. Samples of concentrated coarse, fine, and ultrafine PM, obtained with aerosol concentrators, were studied with regard to their chemical properties and redox activity. Redox activity was highest in the ultrafine fraction, in agreement with results indicating ultrafines were the most potent toward inducing that heme oxygenase expression and depleting intracellular glutathione, which has relevance to induction of oxidative stress. Comparison of the redox activity with chemical composition showed a reasonable correlation of redox activity with elemental carbon ($r^2 = 0.79$), organic carbon ($r^2 = 0.53$), and with benzo[*ghi*]perylene ($r^2 = 0.82$), consistent with species typically found in mobile emission sources.

Keywords: PM; Redox cycling; Dithiothreitol; ROS; DTT; Ultrafine particles; Aerosol concentrators

1. Introduction

There is extensive epidemiological evidence associating airborne particulate matter (PM) with adverse health effects in humans (Pope et al., 2002, 2004; Johnson, 2004; Donaldson et al., 2001). The mechanisms of

*Corresponding author. Center for Environmental & Occupational Health, School of Public Health, University of California Los Angeles, Los Angeles, CA 90095-172, USA. Fax: +1 310 206 9903.
E-mail address: jfroines@ucla.edu (J.R. Froines).

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PM-related health effects are still incompletely understood, but a hypothesis under investigation is that many of the adverse health effects may derive from oxidative stress, initiated by the formation of reactive oxygen species (ROS) within affected cells. There is a growing literature on specific health effects in association with cellular oxidative stress including the ability of PM to induce proinflammatory effects in the nose, the lung, and the cardiovascular system (Li et al., 2003a, b; Gonzalez-Flecha, 2004; Squadrito et al., 2001; Donaldson et al., 2001; Baulig et al., 2003). High levels of ROS cause a change in the redox status of the cell (Schafer and Buettner, 2001), thereby triggering a cascade of events associated with inflammation and, at higher concentrations, apoptosis (Nel et al., 2001). Typically, ROS are formed in cells through the reduction of oxygen by biological reducing agents such as NADH and NADPH, with the catalytic assistance of electron-transfer enzymes and redox-active chemical species such as redox-active organic chemicals and metals (O'Brien, 1991; Dellinger et al., 2001; Brunmark and Cadenas, 1989; Squadrito et al., 2001), and toxicity involving both types of agents has been demonstrated (Kumagai et al., 1997; Veronesi et al., 1999; Wu et al., 1999). PM has been shown to participate in these electron-transfer reactions (Squadrito et al., 2001; Baulig et al., 2003; Vogl and Elstner, 1989).

Kumagai et al. (2002) have demonstrated that the redox-active quinone 9,10-phenanthraquinone can effectively catalyze the transfer of electrons from dithiothreitol (DTT) to oxygen, generating superoxide. When this reaction is monitored under conditions of excess DTT, the rate of DTT consumption is proportional to the concentration of the catalytically active redox-active species in the sample (Fig. 1). We have applied this kinetic analysis to diesel exhaust and PM samples and have found that the catalytic capabilities of each sample correlated with heme oxygenase-1 induction and glutathione depletion (Li et al., 2003b). This induction is a cellular response that is associated with oxidative stress (Li et al., 2000) although the stress response protein is up-regulated by several types of stimuli including oxidative stress. We consider the DTT-based chemical reactivity to be a quantitative probe for assessment of the capacity of a PM sample to catalyze ROS generation which will result in induction of oxidative stress.

In this study, we have examined ambient PM from the Los Angeles Basin (LAB) for their DTT-based redox activity, together with chemical characterization assays, to assess the role of organic species in generating redox activity. PM samples of different sizes were collected in the LAB with a particle concentrator/virtual impactor (Kim et al., 2001a, b) and their redox activity, levels of metals, organic carbon (OC), elemental carbon (EC), inorganic ions, and polycyclic aromatic hydrocarbons (PAHs) determined. The results indicate that the DTT-based measure of redox activity correlates with PM content of OC, EC, and PAHs to a lesser degree.

2. Materials and methods

2.1. Reagents

DTT, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), Tris-HCl, and EDTA were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of the highest grade available.

2.2. Sampling locations

Since the objective of this study was to assess PM redox activity including the role of particle size and chemical characteristics, samples of PM were examined at diverse sites. The samples were size fractionated and collected with the Versatile Aerosol Concentration

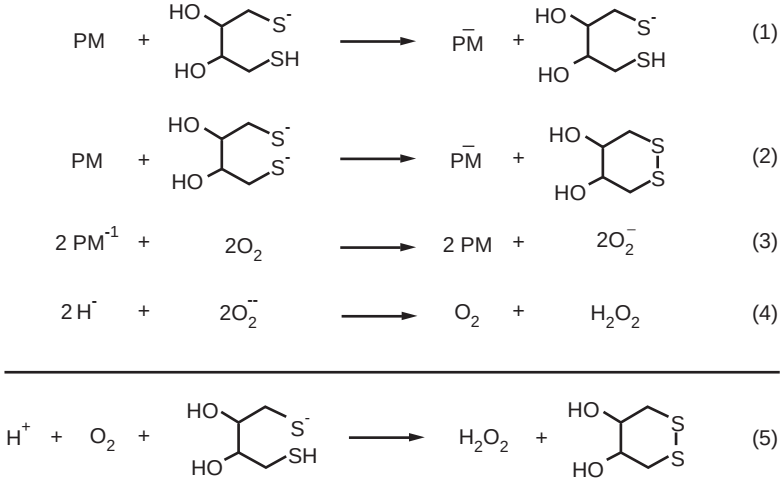

Fig. 1. Chemical reaction between DTT and oxygen with PM as a catalyst.

Enrichment System (VACES) (Kim et al., 2001a, b) at three sites:

(1) Boyle Heights (BH), an area just east of the Los Angeles downtown area. Particles were collected at two locations, 50 m (BH1) and 150 m (BH2) downwind of the confluence of two major freeways (I-60 and I-5) that include heavy diesel traffic.
(2) The main campus of the University of Southern California (USC), which is about 2 km southwest of the downtown area. This site and BH represent classic "source" sites in which aerosols are mostly generated from fresh vehicular emissions.
(3) Claremont (CL), approximately 45 km east and downwind of downtown Los Angeles. This site represents a source as well as a "receptor site," one in which ambient PM originating from emissions in urban Los Angeles have undergone secondary photochemical processes, including gas to particle processes.

2.3. PM sample collection

Ambient coarse (2.5–10 μm), fine plus ultrafine (F/UF) (<2.5 μm), and ultrafine (UF) only (<0.15 μm) particles were collected at the three sites during the period October 2001–January 2003. Particle sampling and collection lasted for 4–7 days and for 6–8 h per day at each site.

Particle collections were conducted by the VACES, using three parallel sampling lines (concentrators) to simultaneously collect coarse and fine particles at a flow rate of 110 L min^{-1}. The VACES is described in greater detail by Kim et al. (2001a, b). Coarse particles were concentrated using a single-nozzle virtual impactor, while fine particles were concentrated by drawing air samples through two parallel lines, using 2.5-μm cut-point preimpactors to remove larger-sized particles. These particles are drawn through a saturation–condensation system that grows particles to 2–3-μm droplets, which are subsequently concentrated by virtual impaction.

Highly concentrated liquid suspensions of these particle modes were obtained by connecting the concentrated output flow from each of the VACES concentrators to a liquid impinger (BioSampler, SKC West Inc., Fullerton, CA). Particles are injected into the BioSampler in a swirling flow pattern so that they can be collected by a combination of inertial and centrifugal forces. This inertia-based collection mechanism, coupled with the short residence time on the order of 0.2 s for particles and gases in the Biosampler, precludes any inadvertent trapping of gaseous copollutants in the particulate layer.

Under normal operating conditions (at its nominal flow rate of 12.5 L min^{-1}), the BioSampler has collection efficiency close to 100% for particles larger than about 1.5 μm. For particles smaller than 1.0 μm, the collection efficiency decreases sharply to less than 50% (Willeke et al., 1998). Operating in conjunction with the VACES, however, the BioSampler can collect any of the PM size ranges with 100% efficiency and at a sampling flow rate that is at least a 10-fold higher than its nominal operating flow rate. Thus, the supersaturational growth of even ultrafine PM to supermicrometer particles enables effective trapping of these particles by the BioSampler and allows us to "concentrate" large numbers of ambient PM into a very small solution of the order of 5–10 mL of water. Before collection of each sample, the BioSampler was autoclaved.

The total amount of particulate loading in the collection medium was determined by multiplying the ambient concentration of each PM mode by the total air sample volume collected by each concentrator line. The particle concentration in the aqueous medium was then calculated by dividing the particle loading by the total volume collected in that time period.

In each sampling line of the concentrator, coarse, fine, and UF PM were concentrated from a flow of 120L min^{-1} to a flow of 6 L min^{-1}, thereby, being enriched by a factor of 20. From the 6 L min^{-1} of the concentrated flows of coarse, fine, and UF PM samples, 4 L min^{-1} was drawn through the BioSampler connected to the respective minor flow, while 2 L min^{-1} passed through a diffusion dryer for fine and UF PM only to remove excess water and dry the aerosol. Diffusion drying of coarse PM was not considered necessary since it is concentrated without hydration of the aerosol. The dry concentrated aerosol flow was then split into two equal halves of 1 L min^{-1}, each diverted into a filter sampler consisting of either a 47-mm Teflon filter (2-μm pore; PTFE Teflon; Gelman Science, Ann Arbor, MI) or a 47 mm prebaked quartz filter (Pallflex Corp., Putnam, CT). The Teflon filters were used to determine particle mass and the metal content, whereas quartz filters were used to determine the PM content of EC, OC, inorganic ions, and PAH. EC and OC were determined using a thermal optical transmittance method as specified in NIOSH method 5040 (Birch and Cary, 1996).

2.4. Chemical analyses

For measurement of mass concentrations, the filters were weighed before and after each field test using a Mettler 5 Microbalance (MT 5, Mettler-Toledo Inc., Highstown, NJ), under controlled relative humidity (40–45%) and temperature (22–24 °C) conditions. At the end of each experiment, filters were stored in the control humidity and temperature room for 24 h prior to weighing to ensure removal of particle-bound water.

Trace elements and metal analysis was performed by Chester Laboratories (Tigard, OR).

The concentration of trace elements and metals was determined by means of X-ray fluorescence subsequent to filter weighing. The quartz filters were cut into two unequal parts, 1/4 and 3/4 of the total filter. The smaller piece was analyzed by means of ion chromatography to determine particle-bound sulfate and nitrate concentrations (Birch and Cary, 1996). Sulfate and nitrate were extracted from these samples ultrasonically for 30 min with Milli-Q deionized water and quantified using a Dionex DX-100 ion chromatograph. NIST traceable standards (SPEX Industries, Edison, NJ) were used as positive control for sulfate (Cat. No. AS-SO49-2y) and nitrate (Cat. No. AS-NO39-2y). A small area (1 cm^2) of the remaining filter (3/4) was removed to determine the EC and OC content of PM.

2.5. PAH analysis

The remaining portion from the filter above was used to determine the concentrations of the PAHs: phenanthrene, anthracene, pyrene, fluoranthene, benz[*a*]anthracene, chrysene, benzo[*ghi*]perylene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, dibenz[*a,h*]anthracene, and indeno[1,2,3-*cd*]pyrene (data not shown) using procedures described elsewhere (Eiguren-Fernandez and Miguel, 2003). In brief, the filters corresponding to each size range were ultrasonically extracted with dichloromethane and the PAH content of the dichloromethane extract was analyzed by HPLC fluorescence using NIST SRM 1649a as the positive control. Standard deviations for triplicate analyses for PAH in this control averaged 8.0% and ranged from 3.8% to 15%.

2.6. DTT assay

Kumagai et al. (2002) have shown that redox-active compounds catalyze the reduction of oxygen to superoxide by DTT, which is oxidized to its disulfide. The remaining thiol is allowed to react with DTNB, generating the mixed disulfide and 5-mercapto-2-nitrobenzoic acid which is determined by its absorption at 412 nm. The PM-dependent DTT consumption is measured under conditions such that the rate is linear, i.e., when less than 20% is depleted. Catalytic activity is expressed as the rate of DTT consumption per minute per microgram of sample less the activity observed in the absence of PM. A sample of a methanol extract of diesel exhaust particles (Cho et al., 2004) is carried through the assay each time as a positive control to monitor the reaction.

In the assay, a Biosampler PM sample of known mass was incubated at 37 °C with DTT (100 μM) in 0.1 M potassium phosphate buffer at pH 7.4 (1 mL total volume) for times varying from 15 to 90 min. The PM samples used here were assayed at concentrations of 5–40 μg mL^{-1}. At the designated times, 1 mL of 10% trichloroacetic acid was added to the incubation mixture and a 0.5-mL aliquot of the reaction mixture mixed with 1 mL of 0.4 M Tris–HCl, pH 8.9 containing 20 mM EDTA and 25 μL of 10 mM DTNB. The concentration of the formed 5-mercapto-2-nitrobenzoic acid was measured by its optical density absorption at 412 nm.

The activity found for the methanol extract of diesel exhaust particles at a concentration equivalent to 5 μg of original particles per milliliter, was 0.09 ± 0.003 nmol of DTT consumed per minute per microgram of sample ($\pm$SD for six replicates). When oxygen consumption was measured under the same conditions, 0.1 nmol was consumed, consistent with a stoichiometry of unity predicted by reaction (5) in Fig. 1. The reaction is completely oxygen dependent as no DTT is consumed under anaerobic conditions (data not shown).

In a study examining the effect of CuII and FeIII on DTT consumption by 9,10-phenanthroquinone at 0.05 μM, addition of equimolar quantities of either compound increased the activity of the quinone by less than 10%, indicating a minimal contribution by metals to this redox process.

The criteria for data reporting on a given sample were DTT consumption less than 25%, a linear rate, and a coefficient of variation (%CV) for triplicates less than 15%. If DTT consumption was greater than 25% or less than 2% or if %CV was greater than 15% for the sample, then a repeat analysis using higher or lower concentrations and shorter or longer time points was performed. Samples in this study were analyzed once as the data met the reporting criteria above. The %CV for the samples reported here ranged from 2% to 14%.

3. Results and discussion

One of the major working hypotheses of the Southern California Particle Center and Supersite has been that PM contributes to adverse cardiorespiratory effects based on their ability to induce oxidative stress. The initial steps in the induction of oxidative stress result from exposure to PM and the subsequent generation of ROS. The ability of PM to generate ROS derives from the chemical constituents including organic molecules and metals in combination with the particle matrix. Accordingly, we are attempting to characterize the particles with regard to their overall activity instead of attempting to isolate compounds of known chemical and biological reactivity. In this context, we used the DTT-based catalytic reduction of oxygen by PM as a measure of their redox activity which may reflect their ability to induce a state of oxidative stress (O'Brien,

1991). This reaction has been demonstrated previously for the highly redox-active quinone 9,10-phenanthraquinone (Kumagai et al., 2002).

The redox activity for PM samples collected at the three sites is shown in Fig. 2. All the PM collected, regardless of site, exhibited activity as measured by the DTT assay. These findings confirm the hypothesis that PM collected in the ambient environment from four different locations in the LAB has redox activity as measured in a nonbiological assay (DTT) for ROS production. This is consistent with the work of Squadrito et al. (2001) who demonstrated that $PM_{2.5}$ is capable of ROS production in a wide range of sites across the U.S.

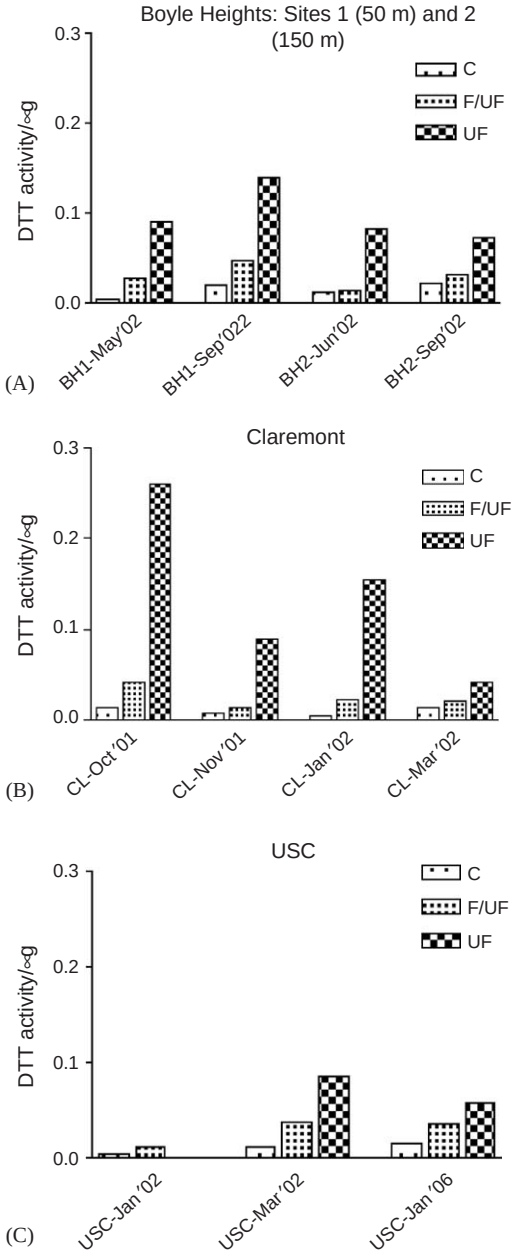

Fig. 2. DTT-based redox activity of PM samples in different locations in the LAB. The activities are shown for the three fractions, coarse, fine/ultrafine, and ultrafine at the locations (BH, A; CL, B; USC, C) at the months and year indicated.

The UF fraction exhibited the greatest redox activity on a per-microgram basis, consistent with the greater potency associated with this fraction observed earlier (Li et al., 2003b) and suggestions by reports in the literature (Oberdorster and Utell, 2002; Utell and Frampton, 2000). The potencies varied with location and with the collection day, but the receptor site had the greatest activity followed by the freeway. Additional investigations will be required to link DTT activity with source, location, and temporal characteristics to more completely characterize PM redox activity in the LAB. The variability was such that it was difficult to establish differences in activity with site of collection. Assessment of changes in the chemistry of PM that may occur between a source and a receptor site would require the samples to be collected simultaneously which we shall address over time.

3.1. Chemical characteristics of concentrated aerosols

For the coarse fractions, the sum of the measured chemical compounds accounted for about 55–90% of the total mass and the concentrations of metals accounted for 35–50% of the total PM mass. The second most abundant coarse PM component was nitrate, accounting for 12–33% of the coarse mass. OC accounted for about 10–25% of the analyzed species, with the highest values generally observed at the freeway locations. This finding is possibly due to the increased amount of direct OC emissions and resuspended road dust associated with the proximity to the nearby freeways.

For the F/UF fraction OC accounted for 25–45% of total mass, with higher concentrations generally observed at the BH and USC sites. OC is by far the most abundant species in UF PM in all sites, accounting for 55–75% of the total mass. EC is the second most abundant species, accounting for about 10–25% of the UF PM mass. Inorganic species account for 10% or less of the UF PM mass. In general, the coarse fractions had the highest content of inorganic chemical species, including transition metals, whereas the UF fractions had the highest content of OC and EC. Consistent with these data, the UF fractions had the highest concentrations of PAH in general. These observations suggest that the coarse fraction is predominantly metal and inorganic ion in content whereas UF fractions were predominantly organic in character. Table 1 lists the PM concentrations in the ambient samples. The UF fraction has the lowest PM concentration in comparison to the coarse and fine/ultrafine fractions.

3.2. Relationship between DTT activity and chemical composition

The consumption of DTT is based on the ability of a given sample to accept electrons from DTT and transfer them to oxygen. A limited number of chemical agents are capable of this reaction including organic compounds such as PAH-quinones (O'Brien, 1991) which are redox cycling agents and act catalytically. Transition metal ions such as those of iron and copper (Gavett et al., 1997; Prahalad et al., 1999) may generate hydroxyl radicals through Fenton chemistry (the metal catalyzed formation of hydroxyl radical from hydrogen peroxide) but are not active in the DTT reaction which measures formation of superoxide anion radicals. The coefficients of determination for OC ($r^2 = 0.53$) and EC ($r^2 = 0.79$) are illustrated in Fig. 3. DTT activity did not correlate with any of the inorganic species, including metals, which is not unexpected since metals, sulfate, and nitrate are inactive in this assay. The greater DTT activity in the UF fraction is consistent with the conclusion that the activity in these samples is due to organic compounds that are not metals or other inorganic species. OC originates from both combustion emissions and secondary atmospheric processes. The correlations suggest that both carbon and organic compounds are important in the redox activity of PM. Schauer (2003) has suggested that EC is a marker for both diesel and gasoline emissions, so the correlation between EC and DTT redox activity suggests that both emission sources, namely diesel and gasoline, may contribute to the activity.

Table 1
Particle concentration in ambient samples ($\mu g\,m^{-3}$)

Location	Coarse	Fine/ultrafine	Ultrafine
Boyle Heights			
Site 1 April'02	23.90	13.91	5.14
Site 1 Sep'02	20.70	31.00	6.04
Site 2 Jun'02	30.90	37.40	5.40
Site 2 Sep'02	18.90	47.10	9.50
Claremont			
Oct'01	9.60	25.70	2.20
Nov'01	7.70	12.30	2.70
Jan'02	14.20	14.10	1.20
Mar'02	5.00	13.50	4.90
USC			
Nov'02	25.90	26.80	1.90
Mar'02	15.90	14.90	3.00
Nov'03	10.90	40.00	9.10

The relative geography of each site is described under materials and methods. The Boyle Heights site 1 is 50 m and its site 2 is 150 m from two freeways. The values represent samples collected over periods of 4–7 days for about 6 h per day by the VACES.

Table 2 shows the values for the coefficient of determination (r^2) between the rate of DTT consumption and the concentration of selected PAHs (expressed in picograms per microgram of PM mass; PPM). The listed PAH were selected for this analysis because they were found in measurable amounts in all samples. The r^2 for PAHs overall was 0.41 and ranged between 0.32 and 0.82, indicative of the variability due to differing source sites. PAHs are not redox active in the DTT assay but must be oxidized to polar compounds including quinones and possibly nitro-PAHs to be redox active. However, PAHs are useful as markers for the source of the organic compounds contributing to redox activity such as quinones. PAH-quinones are generated by the combustion process and we have recently developed a quantitative method for their analysis which was used to demonstrate the presence of at least four quinones in ambient PM samples (Cho et al., 2004). Future studies will determine their airborne concentrations of PAH-quinones in PM and the vapor phase. PAH-quinones are also able to be generated in vivo by biotransformation (Penning et al., 1999).

A stronger association was observed between DTT activity and concentrations of BgP ($r^2 = 0.82$). BgP is a tracer of vehicular combustion emissions, especially gasoline motor vehicles (Marr et al., 1999; Miguel et al., 1998). The modest correlation of the PAHs in general with DTT activity would indicate that the correlation with BgP is reflective of the specific source. The DTT-based redox activity of PM may reflect a contribution

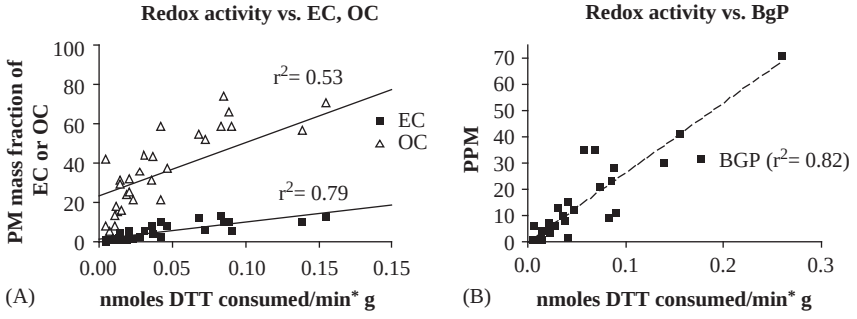

Fig. 3. Correlation diagrams of the redox activity and EC, OC, and benzo[*ghi*]perylene. (PPM units = picograms per microgram of particle mass).

Table 2
Coefficients of determination (r^2) between the rate of DTT consumption and the PAH content of PM samples

Organic species	r^2
Total measured PAH	0.41
Benzo[*g*]perylene (BgP)	0.82
Phenanthrene	0.73
Fluoranthene	0.39
Pyrene	0.73
Benz[*a*]anthracene	0.43
Chrysene	0.60
Benzo[*b*]fluoranthene	0.56
Benzo[*k*]fluoranthene	0.32
Benzo[*a*]pyrene	0.42
Indeno[1,2,3-*cd*]pyrene	0.32

from gasoline motor vehicles. These observations may be relevant to the report of Seagrave et al. (2003) who observed that PM and semivolatile emissions from gasoline engines were more potent than those from diesel engines in eliciting cytotoxic responses in macrophages.

3.3. Exposure vs. sites

When expressed as activity per microgram, redox activity reflects the potency of particles, whereas expression per unit volume of air reflects exposure, i.e., the redox activity to which an individual is exposed. To assess the exposure to redox activity contained in PM, the data of Fig. 2 and Table 1, particle concentrations at the different sites, were used to estimate DTT activity per volume of air. This activity, calculated for the sum of coarse and F/UF fractions, represents the total redox activity of PM10 per volume of air sampled. The results of this estimation are shown in Fig. 4. Note that the exposure to ROS would be greater for the freeway site and USC than for CL even though two of the CL samples had the greatest redox potency (Figs. 2 and 4). The ultrafine fraction is lower in mass concentration and while its potency is considerably greater than the coarse and F/UF fractions, the actual yield of redox activity must be considered across all the size ranges when considering total exposure. We have demonstrated that PM from the ultrafine fraction is able to penetrate cells and lodge in the mitochondria of macrophages and epithelial cells and damage the mitochondria (Li et al., 2003b), whereas the fine and coarse fractions did not demonstrate similar behavior. This latter finding would suggest that the ultrafine fraction may be more important in any health effects that may possibly derive from a mechanism involving ROS and oxidative stress. The precise mechanisms for PM-related health effects are uncertain but the demonstration of the formation of ROS must be considered potentially significant especially since there is evidence of cellular uptake of PM.

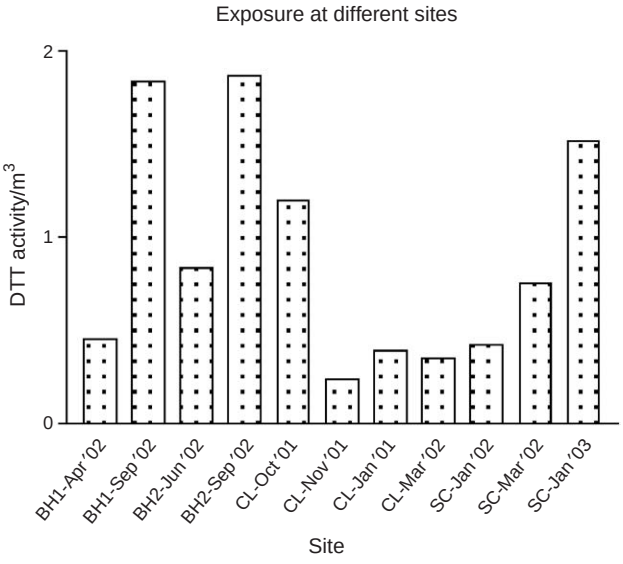

Fig. 4. Exposure at different sites. The bars represent the total redox activity per m^3 at the indicated locations and were generated from the data for Fig. 2 and from the particle concentration values in Table 2.

This study demonstrates that ambient PM samples collected at different sites in the LAB have an inherent capacity to transfer electrons from a source such as DTT to oxygen. The major source of this redox activity appears to be organic compounds associated with gasoline vehicle and diesel emissions as determined by the correlation between DTT activity and OC, EC, and BgP. The greatest DTT potency was seen in the UF fraction of PM, consistent with earlier findings. The results reported here are indicative of the value of a quantitative assay for assessment of redox activity from airborne PM over a wide-ranging geographical area with multiple sources.

The role of metals in PM redox activity requires further investigation since transition metals may undergo Fenton chemistry, producing hydroxyl radical. Thus the possibility exists that redox-active chemicals may interact with metals, both of which are located on PM, to generate hydroxyl radical following PM-catalyzed formation of superoxide anion and hydrogen peroxide. Further evaluation of the spatial and temporal characteristics of PM redox activity are underway. The DTT assay may be a useful tool in determining the relationship between the sources of PM and a chemical property important in their toxicity.

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